Interferon-Gamma Inducible Protein ١٠ (CXCL ١٠) as a Predictor of Liver Fibrosis in Chronic Liver Disease

Thesis

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانكَ لاَ علِمَ لَنَا إلاَّ مَا

عَلَّمْتَنَا إنَّكَ أنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة آية (٣٢)

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List of abbreviations

Ab : Antibody
AFP : Alpha fetoprotein
Ag : Antigen
AH : Alcoholic hepatitis
AIH : Autoimmune hepatitis
ALD : Alcoholic liver disease
ALP : Alkaline phosphatase
ALT : Alanine transaminase
AMP : Adenosine mono-phosphate
ANA : Antinuclear antibodies
Apo-A١ : apolipoprotein A١
AST : Aspartate transaminase
AUC : Area under curve
BCP : Basal core promoter
BCS : Budd–Chiari syndrome
CBC : Complete blood count
CF : Cystic Fibrosis
CFLD : CF liver disease
CL : Chemiluminescence immunoassay
CLD : Chronic liver diseases
COPD : Chronic Obstructive Air Way Diseases
CT : Computerize Tomography
CTGF : Connective tissue growth factor
ECM : Extracelluler matrix
EDHS : Egyptian Demographic Health Survey
EIAs : Enzyme immunoassays
ELISPOT : Enzyme-linked immunosorbent spot
GAD : Glutamic acid decarboxylase
GD : Graves' disease
GGT : Gamma-glutamyltransferase
GSD : Glycogen storage diseases
HBcAg : Hepatitis B core antigen
HBeAg : Hepatitis B e antigen

List of abbreviations *(Cont...)*

HBV : Hepatitis B virus
HCC : Hepatocellular carcinoma
HCV : Hepatitis C virus
HDV : Hepatitis D virus
HE : Hepatic encephalopathy
HMWK : High molecular weight kininogen
HRP : Horseradish peroxidase
HSC : Hepatic stellate cells
HT : Hashimoto's thyroidites
IFN-α : Alfa interferon
INF-γ : Gamma interferon
INF-β : beta interferon
INR : International normalization ratio
ITP : Immune thrombocytopenia
LADA : Latent autoimmune diabetes in adults;
MAPK : Mitogen-activated protein kinase
MELD : Model for end-stage liver disease
MMPs : Metalloproteinases
MMP-۲ : gelatinase-A
MMP-۳ : Stromelysin
MMP-۹ : gelatinase-B
MRI : Magnetic resonance imaging
MS : Multiple sclerosis
NAFLDs : Non-alcoholic fatty liver diseases
NASH : Non-alcoholic steatohepatitis
PBC : Primary biliary cirrhosis
PBMNC : Peripheral blood mononuclear cells
PC : Precore
PICP : Procollagen type I carboxy terminal peptide
PIIINP : Procollagen type III amino-terminal peptide
PT : Prothrombin time
RA : Rheumatoid arthritis
ROC : Receiver Operating Characteristic

List of abbreviations *(Cont...)*

ROS : Reactive oxygen species
RT-PCR : Reverse transcriptase polymerase chain reaction
SGA : Small for gastional age
SGPT : Serum glutamic pyruvate transaminase
SS : Sjogren syndrome
TA : Toxic adenoma
TGF-β١ : Transforming growth factor-β١
TNF-α : Tumor necrosis factor alfa
WD : Wilson disease
YKL-٤٠ : chondrex

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Introduction

Chronic liver diseases (CLD) and its end-stages, whether benign or malignant (cirrhosis and hepatocelluler carcinoma), are leading causes of morbidity and mortality worldwide with enormous socioeconomic costs ***(Dooley and Dijke, ٢٠١١).***

Hepatitis C virus (HCV) infection, the most common cause of CLD, is estimated to affect ١٧٠ million individuals worldwide (٢٪ of the world's population). The recently published Egyptian Demographic Health Survey (EDHS) in ٢٠٠٩ estimated an overall anti-HCV antibody prevalence of ١٤,٧٪. The number of Egyptians estimated to be chronically diseased was ٩,٨٪ (more than ٥٠٠،٠٠٠ new HCV infections occur every year**)** ***(Millera and Abu-Raddad, ٢٠١٠)***.

Of all individuals exposed to the virus, the majority (٥٠٪–٨٠٪) will develop chronic infection that can result in cirrhosis and/or hepatocellular carcinoma. However, the rate of histologic progression of chronic HCV infection varies considerably among patients. Although ٢٠٪–٣٣٪ of infected individuals will experience progression to cirrhosis over ٢٠–٣٠ years, the remainder will have mild chronic hepatitis that either does not progress or progresses very slowly. Thus, identification of patients with accelerated progression is of tremendous importance, because early treatment can prevent cirrhosis and HCC ***(Zeremski et al., ٢٠٠٩).***

In nearly all liver diseases, progression from healthy tissue to cirrhosis is mediated by a chronic inflammatory reaction within the liver parenchyma that activates stellate cells and leads to the excess deposition of extracellular matrix proteins. This inflammatory reaction is considered to be a main predictor of disease progression across different liver disease entities. The recruitment of immune cells into the damaged liver is orchestrated by chemokines, a class of soluble immune mediators with variable chemotactic and cytokine-like functions that altered the architecture of the liver as a result of excessive scarring, development of small nodules, and changes in liver tissue ***(Tacke et al., ٢٠١١).***

For almost all causes of chronic liver disease, assessment of fibrosis is important in estimating the prognosis of and determining the surveillance strategy for liver cancer. Moreover, assessment of fibrosis is an important parameter for decisions of antiviral therapy in viral hepatitis. Liver biopsy is still the standard and most commonly used procedure in the assessment of liver fibrosis. However, it is an invasive method associated with patient discomfort and in rare cases with serious complications ***(El-Shabrawi and Isa, ٢٠١١).*** The limitations of the procedure, including its repeatability and reproducibility, have prompted a search for non-invasive markers of hepatic fibrosis. Non-invasive procedures such as transient elastography (FibroScan) and serum biomarkers (particularly Fibrometre, Fibrotest and Hepascore) have been developed in order to avoid biopsy, however, although significant advances have been achieved in this field, none of the currently available

indices has sufficient accuracy to replace liver biopsy in the assessment of hepatic histology in patients with chronic HCV infection ***(Degos et al., ٢٠١٠).*** The primary limitation of these indices is their inability to identify patients with intermediate stages of fibrosis ***(Zeremski et al., ٢٠٠٩).***

CXCL١٠/ inducible protein-١٠ (IP-١٠) is a secreted polypeptide of ١٠ kDa that was first identified as an early response gene induced after gamma interferon (IFN- γ) treatment in a variety of cells. CXCL١٠/IP-١٠ can also be induced by alfa interferon (IFN-α) and beta interferon (IFN-β) as well as by lipopolysacharide. CXCL١٠/IP-١٠ belongs to the CXC chemokine family. This chemokine is secreted by activated T cells, monocytes, endothelial cells and keratinocytes and exerts chemotactic activity towards human peripheral blood monocytes and activated T lymphocytes. Other functions of CXCL١٠/IP-١٠ include inhibition of angiogenesis, inhibition of hematopoietic progenitor cell, inhibition of tumor cell growth as well as antiviral actions ***(Asensio et al., ٢٠٠١).***

It has been previously reported that CXCL١٠ is expressed in hepatocytes and that serum CXCL١٠ levels are increased in patients with chronic hepatitis especially HCV infection. CXCL١٠ is specifically produced by hepatocytes in inflammatory areas, and may help to recruit T cells to the hepatic lesions in chronic viral hepatitis. These data have tempted researchers to study CXCL١٠ as a potential marker for degree of liver fibrosis ***(Antonelli et al., ٢٠٠٩).***

Aim of the Work

The aim of this thesis is to study the clinical utility of CXCL-١٠ serum level as a marker for prediction of degree of fibrosis in HCV related chronic liver diseases and to correlate its levels with the results of liver biopsy in patients with no evidence of hepatic cirrhosis by ultrasonography or Child-Pugh classification in cirrhotic patients.

I – Chronic Liver Disease

A. Definition:

Chronic liver diseases (CLDs) are defined as the continuity of clinical and biochemical evidence of hepatic dysfunction for longer than six months ***(Suchy, ١٩٩٦).*** Liver cirrhosis is the final stage of many hepatic diseases characterized by chronic cellular destruction which leads to impaired hepatic function and blood flow. The complications of liver cirrhosis are the result of hepatocellular lesion and portal hypertension, the most frequent complications are ascites, spontaneous bacterial peritonitis, hepatic encephalopathy (between ١٠ and ٥٠٪ of patients with cirrhosis will experience an episode of overt hepatic encephalopathy) ***(Lewis and Howdle, ٢٠٠٣)***, gastro- esophageal varices, hypersplenism and hepatocellular carcinoma (HCC) ***(Dioz et al., ٢٠٠٨).*** Moreover, ٤٠-٧٤٪ of patients infected with HCV might develop at least one extrahepatic manifestation during the course of the disease ***(Cacoub et al., ٢٠٠٠).***

B. Causes of Chronic Liver Disease:

١. Hepatitis Viruses:

a. Hepatitis B:

i. Virology:

Hepatitis B virus (HBV) was recognized as a member of hepadnaviridae family which may cause persistent infections in its natural hosts ***(Howard, ١٩٨٦).***